Journal of Cellular Biochemistry Supplement 35:27–35 (2000)

Signal Transduction Pathways and Chromatin Structure in Cancer Cells

Virginia A. Spencer and James R. Davie*

Manitoba Institute of Cell Biology and the Department of Biochemistry and Medical Genetics, University of Manitoba, Winnipeg, Manitoba, Canada R3E OV9

Abstract Molecular mechanisms controlling gene expression include cell shape, mechanical and chemical signal transduction pathways, chromatin remodeling, and DNA methylation. In this article, we will review the contribution of these molecular mechanisms and structural alterations in the malignant transformation of cells. The mechanical signaling pathway consists of the tissue matrix system that links together the three-dimensional skeletal networks, the extracellular matrix, cytoskeleton, and nuclear matrix. The cytoskeleton array is a dynamic system that transmits signals from the cell exterior to nuclear DNA. The composition and function of this mechanical signaling pathway is altered in cancer cells. Chemical signaling pathways such as the Ras/mitogen-activated protein kinase (MAPK) pathway stimulate the activity of kinases that modify transcription factors, histones, and chromatin remodeling factors. Oncoproteins deregulating this signaling pathway set in motion a series of events that cumulate to chromatin remodeling and aberrant gene expression. J. Cell. Biochem. Suppl. 35:27–35, 2000. © 2001 Wiley-Liss, Inc.

Key words: cytoskeleton; nuclear matrix; histone acetylation and phosphorylation; histone acetyltransferase; histone deacetylase; histone kinase; DNA methylation; cancer

EPIGENETIC MECHANISMS REGULATING GENE EXPRESSION

Alterations in epigenetic mechanisms regulating gene expression contribute to the genesis and progression of cancer. Molecular mechanisms controlling gene expression include signal transduction pathways, chromatin remodeling, and DNA methylation. Also, events leading toward changes in the structural organization of a cell's contents influence gene expression. In this article, we will review the contribution of these molecular mechanisms and structural alterations in the malignant transformation of cells.

Grant sponsor: Medical Research Council of Canada; Grant numbers: MT-9186, RO-15183; Grant sponsors: Cancer Care Manitoba; U.S. Army Medical and Material Command Breast Cancer Research Program; Grant number: DAMD 17-001-10319; Grant sponsor: National Cancer Institute of Canada (NCIC) (Canadian Cancer Society).

*Correspondence to: J.R. Davie, Manitoba Institute of Cell Biology, University of Manitoba, Winnipeg, Manitoba, R3E OV9, Canada. E-mail: Davie@cc.umanitoba.ca

Received 10 October 2000; Accepted 11 October 2000

INVOLVEMENT OF THE TISSUE MATRIX IN GENE EXPRESSION

The shape of a cell is governed by a dynamic tissue matrix system that links together three-dimensional skeletal networks from the nuclear matrix (NM), cytoskeleton (CSK), and extracellular matrix (ECM) [Maniotis et al., 1997a]. The NM is the skeletal framework of the nucleus composed of the nuclear-pore lamina, residual nucleoli, and a network of proteins and RNA [Davie et al., 1999]. This structure binds to DNA regions along the chromatin fiber referred to as matrix attachment regions (MARs), and organizes DNA into loop domains [Davie et al., 1999].

The tissue matrix system is believed to form a structural and functional connection between the cell periphery and DNA, establishing a mechanical signaling pathway to transmit signals from the cell's exterior to nuclear DNA. In support of this, disrupting the cytoskeleton with cytochalasin D or acrylamide prevents a cell from interacting with the surrounding ECM [Haier et al., 1999], and pulling a single chromosome out from an interphase cell results in

the removal of all subsequent chromosomes along with the cytoskeleton [Maniotis et al., 1997b]. Furthermore, a mechanical tug of the integrins on the cell surface alters the organization of the cytoskeletal filaments, the location of the nucleoli, and the shape of the nucleus [Maniotis et al., 1997a].

Since the tissue matrix connects the cell periphery with nuclear DNA, any changes in the organization of the components of this system may influence gene expression. For example, a change in cell shape induced by the ECM induces the expression of β-casein [Bissell et al., 1999]. Also, a change in cell shape along with ECM rearrangement leads to changes in the nuclear localization of p53 [Bissell et al., 1999]. Furthermore, the ECM downregulates the expression of TGF-β [Hansen and Bissell, 2000]. Thus, the ECM plays a role in the transcriptional regulation of some genes.

Similarly, changes in the structure and composition of the cytoskeleton may also affect gene expression by influencing the NM structure, and, therefore, DNA organization. Changes in CSK organization have been observed in Kirsten kidney cells when transformed with the *ras* oncogene [Pienta and Coffey, 1992]. In addition, exposure of a cell to the tumor promoter, phorbol 12-tetra-decanoate 13-acetate (TPA), causes gross morphological changes in cell structure and the organization of NM-associated intermediate filaments [Fey and Penman, 1984]. Studies performed in our laboratory show that exposure of the MCF-7 human breast cancer cell line to estradiol increases the total cellular levels of cytokeratins K8, K18, and K19, as well as the levels of cytokeratins K8, K18, and K19 associated with the NM and with nuclear DNA [Davie et al., 1999]. Likewise, the treatment of rat vaginal epithelium with estradiol results in an increase in the levels of total cellular cytokeratins [Kronenberg and Clark, 1985]. Thus, estrogen action appears to involve changes in the cytoskeletal structure. Whether these changes are directly involved in or are a consequence of estrogen-induced gene expression remains to be determined.

Like the intermediate filament arrays, the actin microfilament arrays of the cytoskeleton also appear to play an important role in gene expression. Estradiol treatment of the MCF-7 human breast cancer cell line causes rearrangements in the F-actin cytoskeleton that lead to the formation of lamellipodial structures [DePasquale, 1999]. Furthermore, numerous studies indicate that actin exists in the nucleus, and that nuclear actin plays a role in chromatin remodeling. β-actin has been identified as a component of the chromatin remodeling SWI/SNF-like Brg-associated factor complex. Also, the actin-related proteins, Arp7 and Arp9, are shared components of the SWI/SNF and RSC chromatin remodeling complexes within yeast *Saccharomyces cerevisiae*. In a recent study, the Act1 actin protein, and the Act3/Act4 actin-related proteins were identified as subunits of the H4/H2A histone acetyltransferase, NuA4. The integrity of the NuA4 complex relies on the presence of Act3/Act4. Furthermore, Act3/Act4 could bind to nucleosomes in vitro through the N-terminal domains of histones H3, H4, and H2A [reviewed in Davie and Moniwa, 2000]. These studies combined with observations that actin is tightly associated with the NM, and that actin is located within close proximity of DNA suggest that actin may play a role in the transcriptional process by promoting the binding of histone acetyltransferases or other components of chromatin remodeling complexes to the NM [Rando et al., 2000].

The NM plays an important role in gene expression by structurally organizing nuclear DNA into loop domains. In addition, the NM is associated with a variety of transcription factors and transcriptional machinery [Davie et al., 1999] (Fig. 1). Studies showing that a large percentage of acetyltransferases and deacetylases co-localise to the NM suggest that the NM plays a role in determining the overall structure of loop domains, as well as the structure of individual genes within these domains. In support of this, treatment of cells with an antibody to the NM protein NuMA, disrupts the NM and alters the pattern of histone acetylation [Lelièvre et al., 1998]. Furthermore, the ECM, which is structurally connected to the NM, induces transcription by promoting interaction between enhancer-bound transcription factors, the basal transcriptional machinery, and histone acetyltransferases [Myers et al., 1998].

In addition to playing an important role in gene expression, tissue structure also influences cell cycle progression. Degradation of fibrillar collagen within the ECM causes the

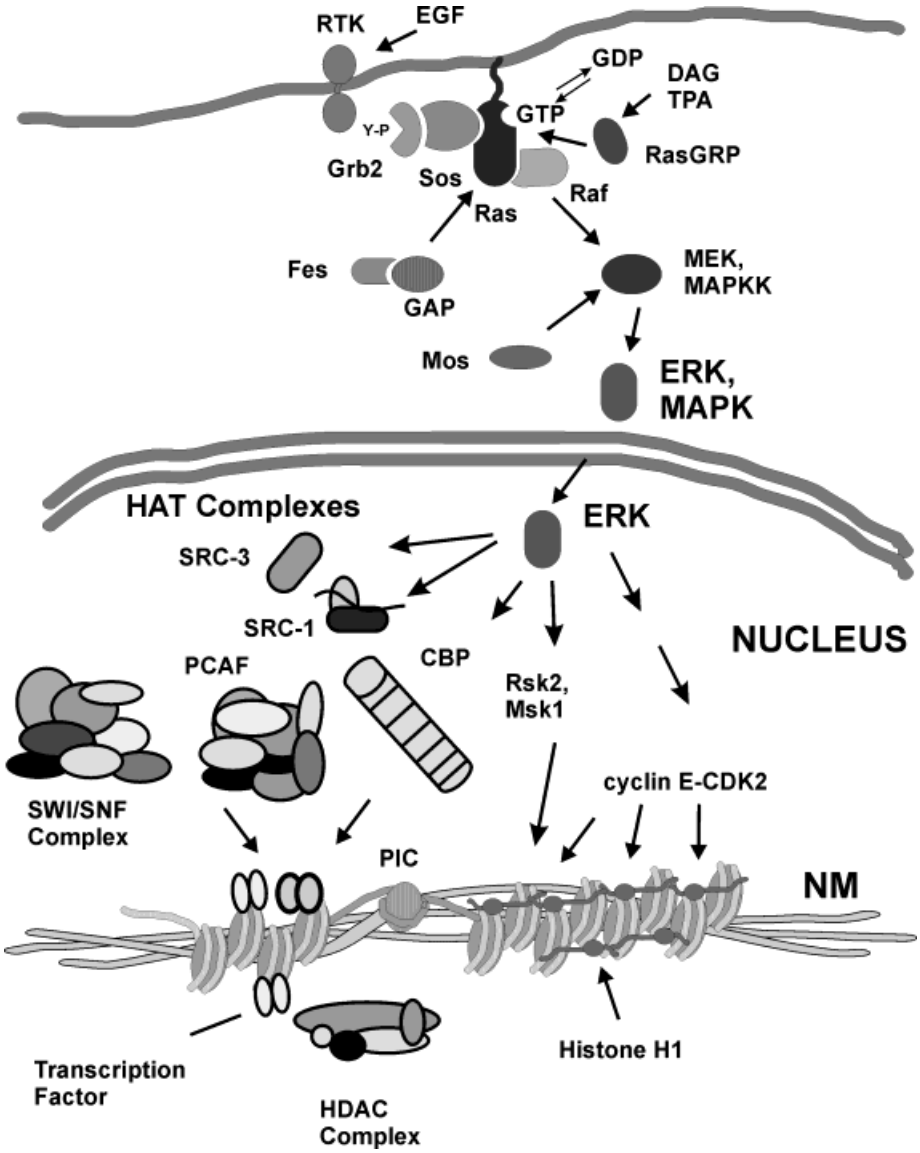

Fig. 1. The Ras/MAPK signaling pathway and the remodeling of chromatin. Stimulation of the Ras/MAPK pathway by EGF or TPA or oncoproteins results in the activation of ERK, which in turn activates Rsk-2, Msk1, histone acetyltransferases (HATs) (CBP, SRC-1, SRC-3), and cyclin E-cdk2 kinase, a histone H1 kinase. In this model Rsk2 and/or Msk1 phosphorylate H3 associated with immediate-early response genes like *c-fos*. H3 and other nucleosomal histones are reversibly acetylated by HATs (CBP, SRC-1, SRC-3, PCAF) and HDACs recruited to the gene. In association with the HATs and HDACs, ATP-driven chromatin remodeling complexes rearrange chromatin structure, resulting in expression of the gene [for review see Davie and Moniwa, 2000]. PIC, preinitiation complex; NM, nuclear matrix.

downregulation of the $p27^{Kip1}$ cell cycle inhibitor in melanoma cells and allows these cells to enter the cell cycle [Henriet et al., 2000]. In addition, limiting the shape of a cell such that it cannot spread out prevents this cell from increasing its cyclin D1 protein levels, phosphorylating the retinoblastoma protein, and decreasing the levels of the $p27^{Kip1}$ cell cycle inhibitor [Huang et al., 1998]. Similarly, disruption of the actin microfilament array prevents G1–S-phase transition [Huang et al., 1998]. Thus, changes in cell shape and cytoskeletal structure most likely play an important role in cell cycle progression by promoting the

interaction of cellular components involved in G1–S-phase transition [Huang et al., 1998].

INVOLVEMENT OF THE TISSUE MATRIX SYSTEM IN ABERRANT GENE EXPRESSION

Considerable evidence suggests that the development of malignancy requires changes in the structure of tissue [Bissell et al., 1999]. The treatment of human breast tumor cells with an inhibitory β-integrin antibody causes these cells to assume a normal phenotype [Hansen and Bissell, 2000]. Similarly, breast tumorigenesis appears to be accompanied by an increase in the expression levels of β-integrin, a protein that helps form the integrin transmembrane receptor complex which connects the cytoskeleton to the ECM [Hansen and Bissell, 2000]. In addition, loss of cell anchorage leads to a decrease in p53 tumor suppressor levels in human foreskin keratinocytes [Bissell et al., 1999]. This decrease, however, was reversed when cells were allowed to reattach to a substratum [Bissell et al., 1999]. Observations that the structure of a tissue is important for cell cycle progression also suggest a role for the tissue matrix in cancer development since alterations in the composition of the tissue matrix system may alter the interactions of cell-cycle regulatory proteins, and contribute toward the abnormal growth of cancer cells [Huang et al., 1998; Henriet et al., 2000].

Changes in NM composition have also been shown to correlate with the metastatic potential of oncogene-transformed mouse fibroblast cell lines and with the state of differentiation of breast cancer cell lines [Davie et al., 1999]. Also, numerous studies show that the NM protein profiles of normal and cancer cells differ [Muenchen and Pienta, 1999; Deppert, 2000; Davie et al., 1999]. Breast carcinomas but not normal or benign cells express a 114-kDa matrix attachment region binding protein, and the expression of this protein is elevated in poorly differentiated breast ductal carcinomas [Galande and Kohwi-Shigematsu, 2000]. Our group has also observed differences in the levels of NM proteins associated with DNA in various breast cancer cell lines representing different stages of breast tumor development [Spencer et al., 2000]. The NM organizes and regulates nuclear processes [Deppert, 2000]. This structure organizes DNA into loop domains as well, [Deppert, 2000]. Because of this, differences in the expression of NM proteins may alter DNA topology, as well as interactions between the NM-associated transcriptional machinery and various genes. Thus, changes in the NM composition may cause genes that are normally inactive to become situated close to the transcriptional machinery. The abnormal expression of a gene could subsequently initiate a series of events linked to cancer development. In support of this, we have recently identified differences in the two-dimension profiles of total and DNA-associated NM proteins isolated from a panel of estrogen receptor positive cell lines reflecting different stages of malignant progression in breast cancer (Spencer, VA, Samuel, SK, and Davie, JR, unpublished). Whether these structural alterations in the NM organization actually contribute toward aberrant gene expression and breast cancer development or are simply a consequence of the transformation process remains to be determined.

The organization of the cytoskeleton also appears to be important in tumor development since the disruption of the microfilament or intermediate filament array alters the metastatic potential of tumor cells [Haier et al., 1999]. In addition, the principal cytokeratins in breast tumors are K8, K18, and K19, whereas cytokeratins K4, K5, K6, K14, and K17 are predominantly expressed in normal breast epithelial cells [Holth et al., 1998]. The majority of breast cancer cells express the estrogen receptor (ER). In their early stage of development, these cells are dependent on estrogen for proliferation. However, as these cells progress to a more malignant stage, they no longer require estrogen for growth [Hansen and Bissell, 2000]. The levels of total, NM-associated and DNA-associated cytokeratins K8, K18, and K19 in hormone-dependent MCF-7 human breast cancer cells are induced by exposure to estradiol. However, this inductive response appears to be lost when these cells are chronically grown in estrogen-deplete conditions [Davie et al., 1999]. Thus, the organization of the intermediate filament array in hormone-dependent human breast cancer cells appears to differ from that in hormone-independent cells [Davie et al., 1999].

The cytoskeletal protein, vimentin, also appears to play an important role in tumorigenesis. Evidence that bone-marrow-derived micrometastatic prostate, breast, lung, and

colon cancer cell lines all contain vimentin suggests a role for this protein in cell motility, growth, and invasion [Putz et al., 1999]. The co-expression of vimentin and cytokeratin intermediate filaments appears only in hormone-independent, and highly invasive human breast cancer cell lines, while hormone-dependent, poorly invasive human breast cancer cell lines do not express vimentin [Kirschmann et al., 1999]. These observations combined with evidence that vimentin and cytokeratins associate with DNA in situ further suggest that the organization of cytokeratin- and vimentin-containing intermediate filament arrays may influence the structure of chromatin such that genes conferring a cell with an invasive phenotype start to become expressed [Davie et al., 1999; Spencer et al., 2000].

NM AND CYTOSKELETAL PROTEINS IN CANCER DIAGNOSIS

Considerable research has been conducted to develop non-invasive methods for cancer detection. The identification of cancer-specific NM proteins has instigated a widespread effort for identifying NM proteins that are specific for cancer cells and easily detected in the blood or serum of cancer patients. From this effort, elevated levels of the bladder cancer-specific NM protein BLCA-4 have been identified in the urine of bladder cancer patients [Konety et al., 2000]. In addition, NMP22 has been identified as a sensitive indicator for bladder cancer that can be detected in the urine of bladder cancer patients. Because of this, a kit detecting NMP22 in urine has now been made available for bladder cancer screening [Konety and Getzenberg, 1999]. Thus, the use of NM proteins as diagnostic indicators for cancer shows great promise. However, it is important to further elucidate the role of these NM proteins in cancer development since this may reveal new targets for cancer therapy.

CHROMATIN REMODELING COMPLEXES

There are two broad groups of chromatin remodeling complexes: complexes that use ATP hydrolysis to increase access of nucleosomal DNA to transcription factors and nuclear enzymes, and histone modifying enzymes, which include histone acetyltransferases (HATs), histones deacetylases (HDACs), and histone kinases [for review see Davie and Moniwa, 2000; Sterner and Berger, 2000] (Fig. 1). HATs, HDACs, and chromatin remodeling (SWI/SNF) complexes are associated with the nuclear matrix [Davie and Moniwa, 2000]. Alterations in NM proteins that are observed in cancer cells likely herald changes in the nuclear location and function of chromatin remodeling complexes and the signals that they are receiving and/or transmitting.

Histone acetyltransferases (HATs) catalyze the addition of acetate to specific lysine residues located in the amino-terminal tails of the nucleosomal histones (H2A, H2B, H3, H4) [Sterner and Berger, 2000]. Most HATs identified to date are transcriptional coactivators, which are recruited to specific promoters and enhancers by transcription factors, and the HATs exist as large multiprotein complexes [Davie and Moniwa, 2000] (Fig. 1). HATs often operate in conjunction with ATP-driven chromatin remodeling complexes. The net result of these activities is the destabilization of higher order chromatin structure and the movement and/or dissolution of nucleosomes, allowing access of the transcription factors and the transcription machinery to specific regulatory sites [for review see Davie and Moniwa, 2000]. Histone deacetylases and chromatin remodeling complexes may also work together in gene silencing. The regulation of these chromatin remodeling complexes is intertwined with signal transduction pathways, which are perturbed in cancer cells.

In humans, loss of one allele of CBP, a HAT, is the underlying defect in the Ruberstein–Tabyi syndrome. Patients with this syndrome are more prone to cancer, consistent with the suggestion that CBP/p300 may function as a tumor suppressor. Further, somatic translocations involving the *CBP* gene are found in various types of hematological malignancies [for review see Blobel, 2000].

The HATs CBP, SRC-1, and SRC-3 are phosphoproteins. CBP/p300, one of the more potent HATs within a mammalian cell, acetylates the four nucleosomal histones and transcription factors [Sterner and Berger, 2000]. CBP is an integrator of several signal transduction pathways (STPs), and many factors are in competition to recruit CBP/p300 [for review see Davie and Chadee, 1998]. CBP is phosphorylated by ERK1, enhancing the HAT activity of CBP in vitro. Thus, the activity of

CBP may be regulated by the Ras-MAPK pathway, the regulation of which is the target of several oncoproteins (Fig. 1). Likewise, AIB1 (amplified in breast cancer 1; also named SRC-3) is phosphorylated by Erk2; this phosphorylation event increases AIB1's affinity for CBP. SRC-1 is also phosphorylated through the mitogen-activated protein kinase (MAPK) pathway [Rowan et al., 2000]. The steroid receptor coactivators SRC-1 and SRC-3 (also called p/CIP, ACTR, RAC3, AIB1, and TRAM-1) are recruited by ligand-bound nuclear receptors (e.g., estradiol-bound estrogen receptor). Thus, the elevated expression of AIB1 coupled with a deregulated Ras-MAPK pathway may result in improper expression of hormone responsive genes [for review see Davie and Moniwa, 2000].

The association of the chromatin modifying and remodeling complexes with the NM is dynamic. As an example, upon the addition of estradiol of breast cancer cells, the ligand-bound estrogen receptor is rapidly recruited to the NM, which in turn recruits the HAT SRC-1 to the NM [Stenoien et al., 2000]. The organization of the cytoskeleton may play an important role in this dynamic attachment. As discussed above, estradiol administration to breast cancer cells results in the reconfiguration of the cytoskeleton, the formation of contacts between intermediate filaments and nuclear DNA, and the genesis of nuclear bodies associated with the NM [Davie et al., 1999; McNeil et al., 2000].

Eight HDACs have been identified in mammalian cells, and several of the HDACs are present in large, multiprotein complexes [Davie and Moniwa, 2000]. PML-RARα, PLZF-RARα and AML-1-ETO, oncoproteins, which are generated by chromosomal translocations, in acute promyelocytic leukemia, recruit HDAC complexes. In addition to instigating the abnormal recruitment of HDAC to specific genes, these chimeric proteins also alter the subnuclear trafficking of the HDACs to different sites on the NM [McNeil et al., 2000]. HDAC complexes are also recruited by the BTB/POZ domain found in the oncoprotein LAZ3/BCL6. The recruitment of HDAC is crucial to the transforming potential of these oncoproteins [for review see Cress and Seto, 2000; Weidle and Grossmann, 2000]. Inhibiting the HDAC activity with a new generation of HDAC inhibitors appears to be a promising approach to the treatment of these cancers [Su et al., 2000; Weidle and Grossmann, 2000].

DNA METHYLATION AND HISTONE DEACETYLATION

DNA methylation is associated with gene silencing, chromosome X-inactivation, and imprinting. The methyl-CpG-binding protein 2 (MeCP2) recruits the HDAC complex Sin3, [Jones et al., 1998] while the methyl-CpG-binding domain-containing protein (MBD2) recruits the HDAC complexes Sin3 and NuRD complexes, providing several avenues for coupling DNA methylation and histone deacetylation in gene silencing. DNMT1, which is the principal enzyme in the maintenance of mammalian DNA methylation, is localized in replication sites, which are associated with the NM. DNMT1 binds to HDAC1 and HDAC2. HDAC1, Rb, and E2F form a complex with DNMT1. The coupling of HDAC1 and HDAC2 with DNMT1 provides a mechanism for temporally coupling histone deacetylation with methylation during replication [for review see Davie and Moniwa, 2000]. Overexpression of DNMT1 transforms cells, while inhibition of DNMT1 activity or expression reverses the transformed phenotype [Rennie and Nelson, 1999; Slack et al., 1999; Knox et al., 2000]. Interestingly, inhibition of DNMT1 expression inhibits DNA replication [Knox et al., 2000].

Deregulation of DNA methylation is observed in cancer cells. Nickel sulfide and nickel subsulfide are potent carcinogens that inhibit histone H4 acetylation and augment DNA methylation [Broday et al., 2000]. It has been suggested that these metal carcinogens induce malignant transformation of cells by silencing the expression of tumor suppressor genes [McBurney, 1999].

HISTONE PHOSPHORYLATION AND CHROMATIN REMODELING IN TRANSFORMED CELLS

Persistent activation of the Ras-MAPK signaling pathway results in elevated levels of phosphorylated histone H1b and histone H3 and a decondensed chromatin structure in oncogene-transformed mouse fibroblasts [Chadee et al., 1999] (Fig. 1). Further, *Rb*-deficient human fibroblasts have increased levels of phosphorylated H1 and a relaxed chromatin

structure [Herrera et al., 1996]. Cyclin E-cyclin-dependent kinase 2 (cdk2) is responsible for increasing the levels of phosphorylated H1. Two kinases, Rsk2 and Msk1, which are activated by the Ras-MAPK pathway, phosphorylate H3.

Phosphorylation of mouse H1b is dependent upon ongoing transcription and replication processes. The inhibition of these processes results in decreased levels of phosphorylated H1b probably by restricting the access of H1b to cyclin E-cdk2 kinase [Davie and Chadee, 1998]. The modification of this mouse histone is unique in this regard. No other histone modification has been shown to be dependent upon these processes.

Activation of the Ras-MAPK pathway results in the rapid phosphorylation of H3 and the transcriptional activation of the early response genes *c-fos* and *c-jun*. We provided the first direct evidence that the newly phosphorylated H3 is associated with induced *c-fos* and *c-myc* genes [Chadee et al., 1999]. Fibroblasts from these Coffin–Lowry patients, who have a mutation in the *Rsk2* gene, do not exhibit phosphorylation of H3 when the Ras-MAPK pathway is stimulated by EGF or TPA, and, interestingly, growth factor-induced expression of the *c-fos* gene is severely impaired. Also H89, an inhibitor of MSK1 but not of ERKs or Rsk2, inhibited EGF-stimulated H3 phosphorylation and expression of *c-fos* and *c-jun*. Following the phosphorylation event, H3 that is associated with the immediate-early response genes (*c-fos* and *c-jun*) is acetylated at lysine 14 [Cheung et al., 2000]. At the time that this article was being prepared, it was not clear which HAT was responsible. However, CBP/p300 is a good candidate [for review see Davie and Moniwa, 2000].

Newly phosphorylated H3 is located in numerous small foci scattered throughout the interphase nuclei of TPA-treated cells. These foci were found outside condensed chromatin regions. Highly acetylated H3 is also observed in similarly positioned numerous small foci, which agrees with the observation that H3 phosphorylation is restricted to a small fraction of H3 histones that are dynamically highly acetylated [Hendzel et al., 1998].

The phosphorylation and acetylation of H3 and perhaps acetylation of the other core histones are likely involved in the induced expression of immediate-early genes by remodeling the chromatin fiber. Consistent with this view, the *c-fos* chromatin becomes more DNAase I-sensitive following activation of the Ras-MAPK pathway. As the H3 tail contributes to the folding and inter-association of chromatin fibers, modification of the H3 tail by acetylation and phosphorylation may destabilize higher order compaction of the chromatin fiber [for review see Davie and Moniwa, 2000]. The decondensation of chromatin by persistent activation of the Ras-MAPK pathway in cancer cells is likely a factor in the aberrant gene expression displayed by these transformed cells.

FUTURE DIRECTIONS

Intervention in the mechanical and chemical signaling pathways results in aberrant gene expression observed in cancer cells. Aberrant nuclear and cellular structures are hallmarks of malignant transformation. Future studies will decide if changes in nuclear organization contribute to or are a consequence of transformation. Accompanying the changes in nuclear structure is the presence of cancer-specific NM proteins. In the diagnosis and prognosis of cancer, the quest to identify informative NM proteins will continue. It is our view that the identification of those NM proteins that are associated with nuclear DNA will be particularly fruitful. There is considerable activity and excitement in unfolding the role of massive, multiprotein chromatin remodeling/modifying complexes in manipulating chromatin structure and function. Future studies will reveal mechanisms involved in altering the activity and targeting of these complexes to chromatin and NM sites in cancer cells. In this review, we have discussed the deregulation of histone acetylation and phosphorylation in cancer cells. Histones are also modified by methylation, ubiquitination, and ADP-ribosylation and these modifications likely alter chromatin structure and function. Future studies will reveal the enzymes that catalyze these modifications and how their activities are sidetracked in cancer cells.

ACKNOWLEDGMENTS

A Medical Research Council of Canada Senior Scientist to J.R.D. and a NCIC Studentship to V.A.S. are gratefully acknowledged.

REFERENCES

Bissell MJ, Weaver VM, Lelievre SA, Wang F, Petersen OW, Schmeichel KL. 1999. Tissue structure, nuclear organization, and gene expression in normal and malignant breast. Cancer Res 59:1757–1763s; discussion 17.

Blobel GA. 2000. CREB-binding protein and p300: molecular integrators of hematopoietic transcription. Blood 95:745–755.

Broday L, Peng W, Kuo MH, Salnikow K, Zoroddu M, Costa M. 2000. Nickel compounds are novel inhibitors of histone H4 acetylation. Cancer Res 60:238–241.

Chadee DN, Hendzel MJ, Tylipski CP, Allis CD, Bazett-Jones DP, Wright JA, Davie JR. 1999. Increased Ser-10 phosphorylation of histone H3 in mitogen-stimulated and oncogene-transformed mouse fibroblasts. J Biol Chem 274:24914–24920.

Cheung P, Tanner KG, Cheung WL, Sassone-Corsi P, Denu JM, Allis CD. 2000. Synergistic coupling of histone H3 phosphorylation and acetylation in response to epidermal growth factor stimulation. Mol Cell 5:905–915.

Cress WD, Seto E. 2000. Histone deacetylases, transcriptional control, and cancer. J Cell Physiol 184:1–16.

Davie JR, Chadee DN. 1998. Regulation and regulatory parameters of histone modifications. J Cell Biochem 30/31:203–213.

Davie JR, Moniwa M. 2000. Control of chromatin remodeling. Crit Rev Eukaryot Gene Expr (in press).

Davie JR, Samuel SK, Spencer VA, Holth LT, Chadee DN, Peltier CP, Sun J-M, Chen HY, Wright JA. 1999. Organization of chromatin in cancer cells: role of signalling pathways. Biochem Cell Biol 77:265–275.

DePasquale JA. 1999. Rearrangement of the F-actin cytoskeleton in estradiol-treated MCF-7 breast carcinoma cells. Histochem Cell Biol 112:341–350.

Deppert W. 2000. The nuclear matrix as a target for viral and cellular oncogenes. Crit Rev Eukaryot Gene Expr 10:45–61.

Fey EG, Penman S. 1984. Tumor promoters induce a specific morphological signature in the nuclear matrix-intermediate filament scaffold of Madin-Darby canine kidney (MDCK) cell colonies. Proc Natl Acad Sci USA 81:4409–4413.

Galande S, Kohwi-Shigematsu T. 2000. Caught in the act: binding of Ku and PARP to MARs reveals novel aspects of their functional interaction. Crit Rev Eukaryot Gene Expr 10:63–72.

Haier J, Nasralla M, Nicolson GL. 1999. Different adhesion properties of highly and poorly metastatic HT-29 colon carcinoma cells with extracellular matrix components: role of integrin expression and cytoskeletal components. Br J Cancer 80:1867–1874.

Hansen RK, Bissell MJ. 2000. Tissue architecture and breast cancer: the role of extracellular matrix and steroid hormones. Endocr Relat Cancer 7:95–113.

Hendzel MJ, Kruhlak MJ, Bazett-Jones DP. 1998. Organization of highly acetylated chromatin around sites of heterogeneous nuclear RNA accumulation. Mol Biol Cell 9:2491–2507.

Henriet P, Zhong ZD, Brooks PC, Weinberg KI, DeClerck YA. 2000. Contact with fibrillar collagen inhibits melanoma cell proliferation by up-regulating p27KIP1 [In Process Citation]. Proc Natl Acad Sci USA 97:10026–10031.

Herrera RE, Chen F, Weinberg RA. 1996. Increased histone H1 phosphorylation and relaxed chromatin structure in *Rb*-deficient fibroblasts. Proc Natl Acad Sci USA 93:11510–11515.

Holth LT, Chadee DN, Spencer VA, Samuel SK, Safneck JR, Davie JR. 1998. Chromatin, nuclear matrix and the cytoskeleton: role of cell structure in neoplastic transformation. Int J Oncol 13:827–837.

Huang S, Chen CS, Ingber DE. 1998. Control of cyclin D1, p27(Kip1), and cell cycle progression in human capillary endothelial cells by cell shape and cytoskeletal tension. Mol Biol Cell 9:3179–3193.

Jones PL, Veenstra GJ, Wade PA, Vermaak D, Kass SU, Landsberger N, Strouboulis J, Wolffe AP. 1998. Methylated DNA and MeCP2 recruit histone deacetylase to repress transcription. Nat Genet 19:187–191.

Kirschmann DA, Seftor EA, Nieva DR, Mariano EA, Hendrix MJ. 1999. Differentially expressed genes associated with the metastatic phenotype in breast cancer. Breast Cancer Res Treat 55:127–136.

Knox JD, Araujo FD, Bigey P, Slack AD, Price GB, Zannis-Hadjopoulos M, Szyf M. 2000. Inhibition of DNA methyltransferase inhibits DNA replication. J Biol Chem 275:17986–17990.

Konety BR, Getzenberg RH. 1999. Nuclear structural proteins as biomarkers of cancer. J Cell Biochem Suppl 32-33:183–191.

Konety BR, Nguyen TS, Brenes G, Sholder A, Lewis N, Bastacky S, Potter DM, Getzenberg RH. 2000. Clinical usefulness of the novel marker BLCA-4 for the detection of bladder cancer. J Urol 164:634–639.

Kronenberg MS, Clark JH. 1985. Changes in keratin expression during the estrogen-mediated differentiation of rat vaginal epithelium. Endocrinology 117:1480–1489.

Lelièvre SA, Weaver VM, Nickerson JA, Larabell CA, Bhaumik A, Petersen OW, Bissell MJ. 1998. Tissue phenotype depends on reciprocal interactions between the extracellular matrix and the structural organization of the nucleus. Proc Natl Acad Sci USA 95:14711–14716.

Maniotis AJ, Chen CS, Ingber DE. 1997a. Demonstration of mechanical connections between integrins, cytoskeleton filaments, and nucleoplasm that stabilize nuclear structure. Proc Natl Acad Sci USA 94:849–854.

Maniotis AJ, Bojanowski K, Ingber DE. 1997b. Mechanical continuity and reversible chromosome disassembly within intact genomes removed from living cells. J Cell Biochem 65:114–130.

McBurney MW. 1999. Gene silencing in the development of cancer. Exp Cell Res 248:25–29.

McNeil S, Javed A, Harrington KS, Lian JB, Stein JL, van Wijnen AJ, Stein GS. 2000. Leukemia-associated AML1/ETO (8;21) chromosomal translocation protein increases the cellular representation of PML bodies. J Cell Biochem 79:103–112.

Muenchen HJ, Pienta KJ. 1999. The role of the nuclear matrix in cancer chemotherapy. Crit Rev Eukaryot Gene Expr 9:337–343.

Myers CA, Schmidhauser C, Mellentin-Michelotti J, Fragoso G, Roskelley CD, Casperson G, Mossi R, Pujuguet P, Hager G, Bissell MJ. 1998. Characterization of BCE-1, a transcriptional enhancer regulated by prolactin and extracellular matrix and modulated by the state of histone acetylation. Mol Cell Biol 18:2184–2195.

Pienta KJ, Coffey DS. 1992. Nuclear–cytoskeletal interactions: evidence for physical connections between the nucleus and cell periphery and their alteration by transformation. J Cell Biochem 49:357–365.

Putz E, Witter K, Offner S, Stosiek P, Zippelius A, Johnson J, Zahn R, Riethmuller G, Pantel K. 1999. Phenotypic characteristics of cell lines derived from disseminated cancer cells in bone marrow of patients with solid epithelial tumors: establishment of working models for human micrometastases. Cancer Res 59:241–248.

Rando OJ, Zhao K, Crabtree GR. 2000. Searching for a function for nuclear actin. Trends Cell Biol 10:92–97.

Rennie PS, Nelson CC. 1999. Epigenetic mechanisms for progression of prostate cancer. Cancer Metastasis Rev 17:401–409.

Rowan BG, Weigel NL, O'Malley BW. 2000. Phosphorylation of steroid receptor coactivator-1. Identification of the phosphorylation sites and phosphorylation through the mitogen- activated protein kinase pathway. J Biol Chem 275:4475–4483.

Slack A, Cervoni N, Pinard M, Szyf M. 1999. DNA methyltransferase is a downstream effector of cellular transformation triggered by simian virus 40 large T antigen. J Biol Chem 274:10105–10112.

Spencer VA, Samuel S, Davie JR. 2000. Nuclear matrix proteins associated with DNA *in situ* in hormone-dependent and hormone-independent human breast cancer cell lines. Cancer Res 60:288–292.

Stenoien DL, Mancini MG, Patel K, Allegretto EA, Smith CL, Mancini MA. 2000. Subnuclear trafficking of estrogen receptor-alpha and steroid receptor coactivator-1. Mol Endocrinol 14:518–534.

Sterner DE, Berger SL. 2000. Acetylation of histones and transcription-related factors. Microbiol Mol Biol Rev 64:435–459.

Su GH, Sohn TA, Ryu B, Kern SE. 2000. A novel histone deacetylase inhibitor identified by high-throughput transcriptional screening of a compound library. Cancer Res 60:3137–3142.

Weidle UH, Grossmann A. 2000. Inhibition of histone deacetylases: a new strategy to target epigenetic modifications for anticancer treatment. Anticancer Res 20:1471–1485.